REMARKS

The pending claims have been rewritten as new claims 33-46 so as to more particularly point out and distinctly claim the subject matter that Applicants view as their invention. Independent claims 33, 37 and 42 have been amended compared to former claims 1, 7, and 25, on which they are based. As redrafted, the independent claims make clear that the inactive Closotridial neurotoxin contains an inactivated neurotoxin light chain containing one or more mutation in the amino acid sequence as compared to the wild type light chain. Additionally, the inactive toxin contains a Clostridial neurotoxin heavy chain. Support for both of these amendments can be found in the specification at *e.g.*, page 6, lines 20-29.

Claims 41 and 46 have been added, and have no counterpart among the canceled claims. These claims specify that the drug or bioactive agent comprises a member of a Markush group of agents. Support for these claims is found *e.g.*, in Table 1, at pages 11- 13 of the specification. Changes in the remaining claims have been made to either correct minor errors or to make the language of the claims consistent.

Compliance with 37 CFR §1.824 and Statements under 37 CFR § 1.821(f) and 1.825(b)

The Examiner has indicated that the computer readable form (CRF) previously submitted with this application contained an error. Applicants regret any inconvenience connected with this error.

Because the Patent Rules regarding sequence listings have been amended between the application filing date and the present, Applicants have complied with the requirement for a corrected CRF under the current provisions of 37 CFR §1.821-1.825. As this would make the format of the CFR sequence information different from that of the paper copy, Applicants have hereby also included with this communication Substitute Sheets of the paper Sequence Listing also formatted in accordance with current Rules 821-825. The information contained on the Substitute Sheets and new computer readable form is identical, and neither contains new matter over the sequence information originally filed with this application.

Amendment to the Specification

The Examiner has also pointed out an inconsistency between the substitute drawings and the Brief Description of the Drawings present in the specification. To correct the inconsistency, Applicants have amended the specification accordingly. No new matter has been added to the specification in connection therewith.

Claim Rejections under 35 U.S.C. § 102

Former claims 1, 2, 7, and 8 were rejected pursuant to 35 U.S.C. § 102(b) over U.S. Patent No. 4,594,336, to Bizzini (hereinafter the '336 patent). As this rejection may be held to apply to the new claims, the rejection is respectfully traversed.

Bizzini discloses a composition said to be capable of transporting a medicine to the central nervous system. The composition consists of a thiolated fragment of the tetanus toxin termed the B-IIb fragment. The immunopurified B-IIb fragment is apparently atoxic in a mouse model at a dose of 1.9 mg. See '336 patent, paragraph bridging columns 5 and 6. The loss of toxicity appears to be by virtue of loss of a portion of the neurotoxin though proteolytic cleavage.

New independent claims 33, 37, and 42, on at least one of which all remaining claims depend, all indicate that the inactive Clostridial neurotoxin comprises an inactive light chain containing a one or more one amino acid sequence mutation. Bizzini does not disclose mutated amino acid sequences; thus it is respectfully submitted that Bizzini cannot anticipate any of the newly rewritten claims.

The Examiner rejected former claims 1,2, 7, and 24 under 35 U.S.C. §102(e) as anticipated by U.S. Patent No. 5,585,100, to Mond et al. (hereinafter the '100 patent). Applicants respectfully traverse this rejection as it may be held to apply to the new claims.

The '100 patent discloses a dual carrier immunogenic construct which may comprise an antigen such as the tetanus toxoid. Tetanus toxoid is partly purified tetanus toxin which is treated with a cross-linking agent such as gluteraldehyde; the cross-linking of the toxin distorts the conformation of the toxin and tends to complex the toxin molecules, which leads to loss of toxicity.

As was the case with Bizzini, Mond does not disclose inactivation of a toxin light chain by the introduction of one or more amino acid sequence mutation (a limitation of each independent claim). As the reference does not disclose each and every element of the newly redrafted claims, the '100 patent cannot properly be held to anticipate any of the pending claims. Applicants therefore respectfully request that the Examiner refrain from reapplication of this ground of rejection.

Former claims 1, 2, 7, 8 and 22-24 were rejected under 35 U.S.C. §102(e) over International Patent Application WO94/00487, to Halpern. Again, this rejection is respectfully traversed to the extent that it should be applied to any of the newly rewritten claims.

Halpern describes an immunogenic proteolytic fragment of the tetanus toxin derived from the carboxyterminus of the heavy chain of the toxin for the construction of vaccines. See, e.g., Halpern, page 3, lines 20-24. Halpern does not describe a composition comprising any portion of the light chain of the toxin; thus Halpern cannot disclose mutation of the light chain, as required by the present independent claims. Thus, this reference cannot anticipate any of the present claims, and Applicants therefore respectfully submit that this rejection is not applicable thereto.

Claim Rejections under 35 U.S.C. § 103

The Examiner rejected prior claims 3 and 4 under 35 U.S.C. § 103(a) over Bizzini in light of Fraenkel-Conrat. Applicants traverse this rejection as it may be held to apply to any of the newly rewritten claims.

The instant invention concerns a protein for the transport of a drug or bioactive agent comprising an inactive, mutated toxin light chain and a toxin heavy chain. The invention is based in large part on the Applicants' discovery "that both the heavy and L[ight] chains of the Clostridial neurotoxins are required for optimal receptor-ligand interaction. In light of this finding, we reasoned that a toxin transporter would advantageously comprise both chains of the dichain molecule." Specification, at page 6, lines 20-23.

Upon consideration of this fact, and of the differences between the applied references and the invention as required under a *Graham v. John Deere* analysis, it can be seen that the invention of the present application cannot be considered obvious in view of Bizzini and Fraenkel-Conrat. Bizzini is drawn to thiolated derivatives of an atoxic fragment of the wild-type tetanus toxin. Thus, in Bizzini the activity of the neurotoxin is abrogated by cleavage of the holotoxin and retention of a nontoxic portion thereof that binds to the surface of the neural cells. Thiolation of the free amino groups of the atoxic B-IIb fragment permits the binding of chemotherapeutic or pharmacological agents to the fragment.

Fraenkel-Conrat discloses experimental point mutation of the tetanus toxin light chain resulting in loss of the light chain's proteolytic activity; when reconstituted with native heavy chain, the resulting mutant species is not lethal to mice.

Neither of these references provides motivation for the combination of one with the other. Bizzini discloses what is said to be an effective composition for the introduction of drugs within neural cells. This composition, which comprises a fragment of the tetanus toxin, involves no mutation of the light chain of the tetanus neurotoxin. Thus, without the teachings of the present specification, the person of ordinary skill in the art, upon reading Bizzini, would not have any reason to consider further modification of Bizzini's BIIb fragment.

Likewise, Fraenkel-Conrat is concerned with enzymological dissection of the proteolytic activity of the tetanus toxin light chain, and nothing in this reference would suggest compositions for the transport of drugs.

Thus, neither reference alone suggests the presently claimed invention. Neither do the references provide motivation to the person of ordinary skill for their combination.

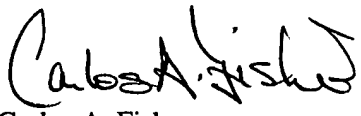
Even assuming *arguendo* that the skilled person were motivated to combine Bizzini and Fraenkel-Conrat, such a combination does not suggest the present invention. Fraenkel-Conrat suggests that the light chain is responsible for neurotoxicity; it was common belief that only the heavy chain of tetanus toxin was responsible for cell surface binding and translocation of the protein across membranes. See e.g., Halpern, page 3, lines 13-17. Thus, the combination of Bizzini and Fraenkel-Conrat, at best, would suggest the use of the heavy chain alone as the carrier of therapeutic agents – there is no suggestion in these combined references why one would wish to go to the trouble of mutating the light chain, much less use any portion of the light chain. Such a suggestion is only provided in the present specification, as indicated above.

The Applicants therefore believe that the present claims are in condition for allowance, and respectfully request that the Examiner issue a Notice to that effect.

Enclosed herewith is a Request for Filing a Continued Prosecution Application containing an authorization to use our Deposit Account 01-0885 for the payment of any fees due in connection

therewith. Should any further fee be due concerning this Amendment, the Commissioner is hereby authorized to use said Deposit Account for the payment thereof.

Respectfully submitted,



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H 2 composition 1

34. The ~~modified toxin~~ of claim 33, wherein said inactive neurotoxin comprises an inactive form of a toxin selected from the group consisting of: tetanus toxin, botulinum toxin A, botulinum toxin B, botulinum toxin C, botulinum toxin D, botulinum toxin E, botulinum toxin F, and botulinum toxin G.

H 3 composition 2

35. The ~~modified toxin~~ of claim 34 wherein said inactive Clostridial neurotoxin is selected from the group consisting of a tetanus toxin comprising a modification of Glu^{224}, a botulinum A toxin comprising a modification at His^{227}, a botulinum A toxin comprising a modification at Glu^{224}, a botulinum toxin other than botulinum toxin A comprising a modification at a site corresponding to His^{227} of botulinum toxin A, and a botulinum toxin other than botulinum toxin A comprising a modification at a site corresponding to Glu^{224} of botulinum toxin A.

36. A pharmaceutical composition for treatment of a neuromuscular dysfunction in a mammal, comprising:

a) an inactive Clostridial neurotoxin comprising

i) an inactivated light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, and

ii) a Clostridial neurotoxin heavy chain,

which inactive neurotoxin has binding specificity for a target nerve cell; and

b) a drug or other bioactive molecule joined to said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell, and

c) a pharmaceutically acceptable excipient.

5 4

37. The pharmaceutical composition of claim 36 wherein said neuromuscular dysfunction is characterized by uncontrollable muscle spasms.

H 6 composition 1 4

38. The ~~modified toxin~~ of either of claims 35 or 36 wherein said drug or other bioactive molecule is an inhibitor of neurotransmitter release.

39. The modified toxin of claim 38 wherein said inhibitor of neurotransmitter release is an inhibitor of a protein selected from the group consisting of SNAP-25, VAMP, and synaptobrevin.

H 8 ~~40~~. The composition ~~modified toxin~~ of either of claims 1 ~~33~~ or 5 ~~37~~ wherein said drug or other bioactive molecule is an active ingredient for treatment of botulism or tetanus.

H 9 ~~41~~. The composition ~~modified toxin~~ of either of claims 1 ~~33~~ or 5 ~~37~~ wherein said drug or other bioactive molecule is selected from the group consisting of:

a) a GABA agonist,
b) a neuronal calcium channel agonist,
c) an adenosine agonist,
d) a glutamate antagonist,
e) a protein synthesis toxin,
f) a zinc-dependent protease inhibitor,
g) a neuronal growth factor,
h) an antiviral agent,
i) a nicotinic antagonist,
j) a neuronal calcium channel blocker,
k) an acetylcholine esterase inhibitor,
l) a potassium channel activator,
m) vasamicol or a vasamicol inhibitor,
n) a ribozyme, and
o) a transcribable gene.

42. A method for treating a mammal having acute botulinum poisoning, comprising:
introducing into said mammal an effective quantity of a pharmaceutically active solution comprising

a) an inactive Clostridial neurotoxin comprising
   i) an inactivated light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species and
   ii) a Clostridial neurotoxin heavy chain,
   which inactive neurotoxin has binding specificity for a target nerve cell; and

b) a drug or other bioactive molecule attached to said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell., thereby lessening the effects of said acute botulinum poisoning.

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~~43~~. The method of claim ~~42~~ 10 wherein said drug or other bioactive molecule is an inhibitor of neurotransmitter release.

44. The method of claim 43 wherein said inhibitor of neurotransmitter release is an inhibitor of a protein selected from the group consisting of SNAP-25, VAMP, and synaptobrevin.

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~~45~~. The method of claim ~~42~~ 10 wherein said drug or other bioactive molecule is an active ingredient for treatment of botulism or tetanus.

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~~46~~. The method of claim ~~42~~ 10 wherein said drug or other bioactive molecule is selected from the group consisting of:

a) a GABA agonist,
b) a neuronal calcium channel agonist,
c) an adenosine agonist,
d) a glutamate antagonist,
e) a protein synthesis toxin,
f) a zinc-dependent protease inhibitor,
g) a neuronal growth factor,
h) an antiviral agent,
i) a nicotinic antagonist,
j) a neuronal calcium channel blocker,
k) an acetylcholine esterase inhibitor,
l) a potassium channel activator,
m) vasamicol or a vasamicol inhibitor,
n) a ribozyme, and
o) a transcribable gene.

ii) an unaltered Clostridial neurotoxin heavy chain[,] which [inactive neurotoxin] has binding specificity for a target nerve cell; and

b) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell.

36. (Amended) A pharmaceutical composition for treatment of a neuromuscular dysfunction in a mammal, comprising:

a) an inactive Clostridial neurotoxin comprising

i) an inactivated light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, and

ii) an unaltered Clostridial neurotoxin heavy chain[,]which [inactive neurotoxin] has binding specificity for a target nerve cell; and

b) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell, and a pharmaceutically acceptable excipient.

39. (Amended) The ~~modified toxin~~ composition of claim ~~38~~ 6 wherein said inhibitor of neurotransmitter release is an inhibitor of a protein selected from the group consisting of [SNAP-25] synaptosome associated protein of molecular weight 25 kDA, [VAMP,] syntaxin, cellubrevin and synaptobrevin.

42. (Amended) A method for treating a mammal having acute botulinum poisoning, comprising:

introducing into said mammal an effective quantity of a pharmaceutically active solution comprising

a) an inactive Clostridial neurotoxin comprising

i) an inactivated light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species and

ii) an unaltered Clostridial neurotoxin heavy chain[,]which [inactive neurotoxin] has binding specificity for a target nerve cell; and

b) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell., thereby lessening the effects of said acute botulinum poisoning.

~~44.~~ (Amended) The method of claim ~~43~~ wherein said inhibitor of neurotransmitter release is an inhibitor of a protein selected from the group consisting of [SNAP-25] synaptosome associated protein of molecular weight 25 kDA, [VAMP,] syntaxin, cellubrevin and synaptobrevin.

REMARKS

Applicants thank Examiner Minnifield for the courtesy extended to Applicants' representative during a personal interview on August 6, 1999. In accordance with the discussion during the interview, Applicants have amended the claims to clearly indicate that the drug or other bioactive molecule is joined to the inactivated light chain of the inactivate neurotoxin, and that the heavy chain is unaltered. Support for these amendments can be found in the specification at e.g., page 6, lines 20-21, and page 14, lines 24-25, respectively.

Additionally, claims 39 and 44 have been amended to replace the acronym "SNAP-25" with the name --synaptosome associated protein of molecular weight 25 kDA--, to delete the term "VAMP", and to add the terms –syntaxin-- and –cellubrevin--. Support for the additions can be found on page 1 of the specification at lines19, and 30-31, respectively.